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Atmospheric and geogenic CO_2 within the crown and root of spruce (*Picea abies* L. Karst.) growing in a mofette area

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ABSTRACT

Mofettes are often investigated in ecology, either as extreme sites, natural analogues to future conditions under climate change, or model ecosystems for environmental impact assessments of carbon capture and storage systems. Much of this research, however, inadequately addresses the complexity of the gas environment at these sites, mainly focusing on aboveground CO_2-enrichment. In the current research, the gaseous environment of Norway spruce (*Picea abies* (L) Karst.) trees growing at the Stavešinske slepice mofette (NE Slovenia) were studied by measuring both soil ($[CO_2]_{soil}$) and atmospheric CO_2 concentrations ($[CO_2]_{air}$). Within the studied site (800 m^2), soil CO_2 enrichment was spatially heterogeneous; about 25% of the area was characterized by very high $[CO_2]_{soil}$ (> 40%) and hypoxic conditions. Aboveground gas measurements along vertical profiles not only revealed substantially elevated $[CO_2]_{air}$ close to the ground (height up to 1.5 m), but also in the upper heights (20–25 m; crown layer). On the basis $\delta^{13}C$ of CO_2, it was shown that elevated CO_2 relates to a geogenic source. Trees grown in high $[CO_2]_{soil}$ were characterized by decreased radial growth; the $\delta^{13}C$ of their wood was less negative than in trees growing in normal soil. Unfavorable gaseous soil conditions should generally be accepted as being by far the most important factor affecting (i.e. disturbing) the growth of mofette trees.

1. Introduction

Carbon degassing from the lithosphere is assumed to play only minor role - with respect to the biological and anthropogenic fluxes - in the global atmospheric C budget and climate effects (Mörner and Etiope, 2002; Gerlach, 2011). Emanations of geogenic carbon have, however, significant and sometimes drastic effect on local atmospheric environment (Grace and van Gardingen, 1997; Kies et al., 2015). At mofettes, sites where CO_2 of deep mantle origin is degassing at ambient temperature, the close surrounding of the vents is characterized by very high and temporally variable air CO_2 concentrations and, very frequently, extremely CO_2-enriched and hypoxic soil (Pfanz et al., 2004). Scientifically, mofettes are used as natural analogues to simulate future conditions under climate change, as model ecosystems for environmental impact assessments for carbon capture and storage systems, and for the investigation of physiological, ecological and evolutionary studies of a range of phylogenetically distinct groups of organisms (Maček et al., 2016). Since CO_2 is a photosynthetic substrate and due to importance of photosynthesis in global carbon cycling, major attention in this research has been devoted to plant performance under naturally elevated CO_2.

Mofette research was strongly stimulated by the global change issue. In the period when FACE experiments were just initiated and had not yet yielded several-years' results, there was a keen interest in obtaining insight into the effects of long-term CO_2 exposure. At the sites with emanations of CO_2, the natural vegetation around the vents is supposed to have a multiple-generation or at least a life-time exposure to CO_2 enrichment (Raschi et al., 1997; Pfanz et al., 2004). Mofette research was intensified in the early 1990s and included studies of growth and physiology of different plant species including grasses (Vodnik et al., 2002; Pfanz et al., 2007), trees (Blaschke et al., 2001; Tognetti et al., 1998, 1999, 2000a, 2007; Vejpustkova et al., 2016), shrubs (Tognetti et al., 2000b, 2001; Kaligarič et al., 2008) and crop plants (Vodnik et al., 2005). These studies have contributed significant knowledge about CO_2 responses and simultaneously revealed certain inconsistencies in the results as well as the difficulties inevitable to experimentation at mofettes (Paoletti et al., 2005); some of these will also be discussed later in this paper.

The research of trees was of particular interest since their growth response to CO_2 enrichment is a significant factor in the global carbon

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cycle. The majority of data are on Mediterranean forests covering CO_2 springs in central Italy (Bossoleto, Lajatico). There, the foliar metabolism and growth of mofette grown oak species trees were thoroughly studied by several authors. Tognetti et al. (1998) reported that stomatal conductivity and transpiration of *Q. pubescens* and *Q. ilex* are reduced upon natural CO_2 enrichment (500–1000 μmol mol^{-1}) which is consistent with general stomatal response of mofette plant species (Badiani et al., 1999; Pfanz et al., 2004). Oak photosynthesis was not always increased, but in contrast Stylinski et al. (2000) reported a substantial promotion of photosynthesis in *Q. pubescens*, without any significant effect on stomatal conductivity and leaf biochemistry. None of the studies indicates downward photosynthetic acclimation in trees with chronic CO_2 enrichment. *Q. ilex* and *Q. pubescens* were also studied by tree ring analyses, which enable an insight into the long-term effect of environmental factors. Hättenschwiler et al. (1997) analyzed tree ring chronologies of *Q. ilex* from two Italian mofettes (lifetime exposure to ca. 650 μmol mol^{-1}) and nearby control sites. Elevated CO_2 had a beneficial effect on water relation in drought periods and increased growth of trees in their juvenile phase, which suggest (from a global change perspective) that the development of young forest stands could be accelerated by elevated CO_2. Growth enhancement was also found in the study of Vejpustkova et al. (2016), who studied the radial growth of poplar trees exposed to different levels of soil CO_2 enrichment. Positive growth effects were, however, not confirmed by Tognetti et al. (2000b) who analyzed radial growth of different woody species (*Arbutus unedo*, *Fraxinus ornus*, *Quercus cerris* and *Quercus pubescens*) on the "I Borboi" mofette near Lajatico in Italy.

There can be different causes for the discrepancies found in these studies. It seems that the major cause could be the problem of accuracy in defining the CO_2-exposure of tested plants, which is a general problem in mofette research (Paoletti et al., 2005). The atmosphere around CO_2-releasing vents is characterized by strong and fast fluctuations of CO_2 concentration; short-term CO_2 measurements with IR-analyzers or absorptive diffusion tubes do not allow the precise determination of the average gas regime. This was confirmed by the study of Saurer et al. (2003) who analyzed ^{14}C, ^{13}C and ^{18}O isotope variations in the rings of *Quercus ilex* in order to assess carbon uptake from natural CO_2 spring to woody biomass. Their study showed that the trees at a high CO_2 location (about 700 μmol mol^{-1}) had been exposed to natural CO_2-enrichment, which had decreased over a 50-year period (1950–2000). The most likely explanations for this temporal trend are a decrease in the source strength or a concentration gradient as the tree becomes higher and crown more distant from the source. In both cases, the trees would have been exposed to higher CO_2 concentrations when they were young. Secondly, it was revealed that the locations that have been selected as controls on the basis of instantaneous atmospheric CO_2 measurements can be significantly influenced by CO_2 from the mofette. This raises an issue of proper controls in mofette experimentation (Paoletti et al., 2005).

In addition to atmospheric gaseous conditions, there could be other reasons for inconsistent or even conflicting results. Studies on grassland mofettes revealed a high level of soil CO_2 enrichment (Pfanz et al., 2004; Vodnik et al., 2006, 2009). High soil CO_2 concentration can induce hypoxia and change soil chemical properties such as soil pH and availability of mineral nutrients (Saßmannshausen, 2010; Rennert and Pfanz, 2016; Maček et al., 2016); it influences soil microbiota (Videmšek et al., 2009; Maček et al., 2011) and can even impair root functioning (Maček et al., 2005). These effects can translate into an insufficient supply of mineral nutrients to shoots, as well as reduced photosynthesis and growth (Pfanz et al., 2007). Hence, when simulating global change scenarios, belowground CO_2 enrichment should be excluded. Unfortunately, many studies neglected this issue focusing on aboveground effects.

The research in this paper was focused on studying the gaseous environment, CO_2 exposure and performance of trees growing at a mofette in a temperate climate (Stavešinci, NE Slovenia). It is not directly related to the issue of global climate change but rather examines the mofettes as sites with unique and frequently extreme environmental conditions. The main aim was to elucidate what is the CO_2 enrichment of the mofette soil and canopy atmosphere at different parts of the studied area, a plot of ca. 800 m^2 around gas-releasing vents. Norway spruce (*Picea abies* (L.) Karsten) trees of different age, growing at different locations around the mofette were examined for CO_2-exposure. The aim was to study the decades-long growth response of those trees with respect to soil and air gas regime.

2. Material and methods

The study was performed in 2012–2014 at the Stavešinske slepice mofette (N46.623685, E15.982226), located in the forest close to the village Stavešinci (NE Slovenia) where a meadow mofette has been thoroughly studied since 2005 (see Maček et al., 2016 and references cited herein). The broader area is characterized by high geothermal activity; mofettes around Stavešinci are known for pure CO_2 emanations (Pfanz et al., 2004; Gabor and Rman, 2016).

Mofette Stavešinske slepice is in the area covered by mixed forest. The most abundant broadleaf species there are *Fagus sylvatica*, *Carpinus betulus, Quercus robur*, *Acer pseudoplatanus*, *Castanea sativa*, etc. Among conifers, there are *Pinus sylvestris and Picea abies*. The latter also dominates, together with *Frangula alnus*, the close surrounding of mofette vents. There are two main vents, pond-like springs with a surface area of ca 1.5 m^2. The Stavešinske slepice mofette has been studied before; however, other than contributions by Pezdič et al. (1998) and Gabor and Rman (2016), there has been no other scientific report on research at this site.

Our research focused on the area of ca. 800 m^2 surrounding two main CO_2-releasing vents (Fig. 1). The spatial distributions of spruce trees growing in this area were measured.

Fig. 1. Stavešinske slepice mofette – main vents (upper panel) and experimental trees (lower panel).

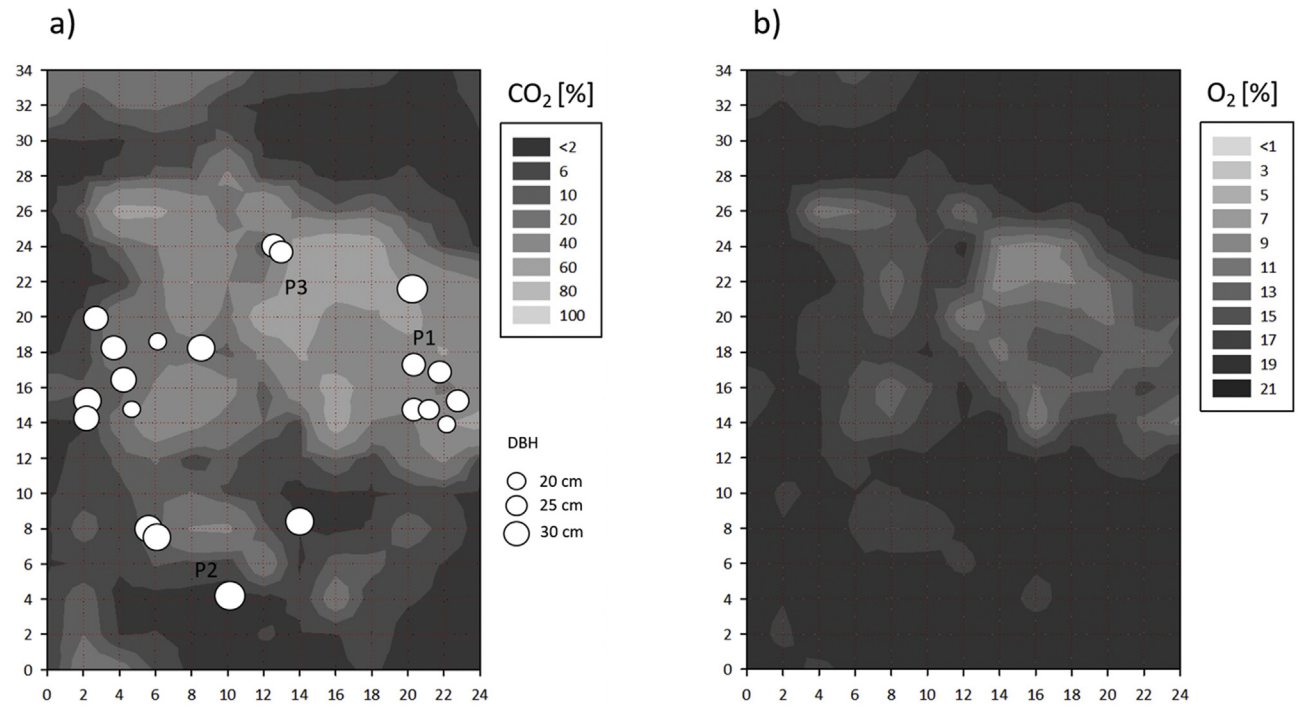

Fig. 2. Soil CO_2 (a) and O_2 (b) concentrations at Stavešinske slepice mofette and positions of spruce (*Picea abies* (L.) Karst.) trees with corresponding diameter of the trunk at breast height (DBH) (a). CO_2 in soil air was measured at different depths (20, 40 and 60 cm) and averaged. P1-3 indicates the sites where atmospheric gas CO_2 concentration was analyzed along vertical profile.

2.1. Gas measurements

Soil gas measurements were done by using a portable landfill gas analyzer (GA2000, Ansyco, Germany) as described by Pfanz et al. (2004). To assess the general gaseous conditions of the studied area, measurements were made on a regular sampling grid with a resolution of 2 m. Altogether, there were 204 sampling locations. At each of these, consecutive drilling to 20, 40, and 60 cm depth was done. From each depth, gas was sampled with the analyzer, and CO_2 and O_2 concentrations were determined. The three concentrations obtained for soil profile were then averaged. Spatial maps of CO_2 and O_2 were created (Fig. 2).

Secondly, a similar soil gas measurement were made around each of the 22 mature spruce trees in the area. At each tree, there were three sampling points equally distributed (distance 120°) in a circle 0.5 m distant from the base of the stem. At each point, the soil air was sampled with the GA2000 analyzer from depths of −10, −20, −40, and −60 cm.

According to previous reports, CO_2 in non-mofette soils rarely exceed concentrations of 3% (Pfanz et al., 2004; Jassal et al., 2005; Vodnik et al., 2006, 2009; Saβmannshausen, 2010). On this basis we marked all trees grown at locations where $[CO_2]_{soil}$ did not exceed the 3% level as *low CO_2 trees*. Trees growing in soils with $[CO_2]_{soil}$ 3–15% and > 15% were marked as *medium CO_2 trees* and *high CO_2 trees*, respectively.

Measurements in the air were made by using an automated sampling system connected to a Li-840A (LiCor, Lincoln, NE USA) infrared gas analyzer. We focused on vertical profiles at two mature spruce trees (trees 1 and 21 in Table 1); one exposed to soil CO_2 enrichment ("high soil CO_2"; tree S21, P1 on Fig. 2) and another for which soil CO_2 concentration was in the range typical for non-mofette soil (see Pfanz et al., 2004) ("low soil CO_2", tree S1, P2 on Fig. 2). PVC tubes with 4 mm diameter and appropriate length were installed to the trunks of both trees to sample air from different heights; ground level, 1.3, 5, 10, 15, 20, and 25 meters above. The highest sampling point was close to the top of the crown (20 m in "high soil CO_2 tree"; 25 m in "low soil CO_2 tree"). Additionally, there was another sampling tubing with a 1 dcl PVC water trap inserted into the soil (depth 0.3 m) close to the trunk. Altogether, there were seven sampling points for vertical gradient at "high soil CO_2" location and eight sampling locations for "low soil CO_2" location. Air was pumped for 2 min with a flow rate of 1 L per minute from one sampling point through the multiplexing system to the analyzer before recording CO_2 concentration. This was enough to clean the volume of the longest sampling tube, multiplexer valve, pump, and sampling cell (approximately 0.5 liter). Consecutive samplings from all sampling points were done within 30 min, and the next sampling cycle then started every full hour. Continuous measurements of two profiles were made in the period from 24 September 2013 to 2 October 2013.

Thereafter, lower profiles were sampled close to young ca. 1 m tall spruce trees, which grow close to the main Stavešinske slepice vent (P3 on Fig. 2). Altogether, there were four profiles and at each air was sampled from the heights of 0.1, 0.3, 0.5, and 0.7 m above the ground. These measurements were done from 2 October 2013 to 10 October 2013.

2.2. Isotopic analyses of the air

Air from all profiles was sampled for $\delta^{13}C$ analysis. There were two sampling campaigns: one early in the morning under calm atmospheric conditions (6:00, indicated as "night") and another during the day (15:00, "day"). High profiles were sampled on October 2, 2013, and low profiles were sampled on October 10, 2013. A syringe, connected to the piping via a T-piece connection, in front of the Li-840A analyzer, was used to sample ca. 50 ml of air. Sampling was performed during the last 30 s of pumping from a single location. The sample was transferred immediately to a pre-evacuated 12-ml glass serum vial. The septum penetration needles allow for direct delivery of the gas sample into the evacuated serum vial, minimizing the possibility of atmospheric contamination (Torn et al., 2003). Similarly, air was sampled directly at the source (main vent of Stavešinske slepice) from a 5-L static chamber which had been left on the surface of the pond for 0.5 h before sampling.

Table 1
Soil CO_2 and O2 in the rooting horizon of spruce (*Picea abies* (L.) Karsten) trees at Stavešinske slepice mofette. $[CO_2]_{soil}$ was measured at different depths. Averaged value for vertical profile was considered when ranging trees according to their soil CO_2 gas regime. DBH = stem diameter at breast height.

tree	DBH [cm]	Soil CO_2/O_2 concentration [%]					soil CO_2
		−10 cm	- 20 cm	- 40 cm	−60 cm	average	
S1	32.0	0.1/20,5	0.1/20,4	0.2/20,4	0.3/20.4	0.2/20.4	low
S2	28.0	0.1/20.4	0.3/20.4	0.8/20.3	1.8/20.1	0.8/20.3	low
S3	28.0	0.3/20.2	0.4/20.2	1.6/20.0	4.0/19.4	1.6/20.0	low
S4	31.5	0.6/20.4	0.9/20.2	2.0/19.9	3.0/19.8	1.6/20.1	low
S5	32.0	0.4/20.5	0.7/20.4	2.1/20.1	3.5/19.8	1.7/20.2	low
S6	31.5	0.7/20.4	1.3/20.2	3.5/19.8	6.5/19.0	3.0/19.9	low
S7	30.0	0.4/20.5	0.9/20.4	3.8/19.8	10.5/18.4	3.9/19.8	medium
S8	21.0	0.6/20.5	0.9/20.4	5.8/19.4	9.0/18.7	4.1/19.8	medium
S9	33.0	0.4/20.3	0.9/20.3	6.3/19.2	11.4/18.2	4.8/19.5	medium
S10	28.5	0.7/20.4	1.7/20.2	7.1/19.1	13.9/17.6	5.9/19.3	medium
S11	20.5	0.8/20.4	1.5/20.2	9.4/18.7	19.5/16.7	7.8/19.0	medium
S12	26.0	2.8/20.0	5.4/19.4	7.0/19.0	16.3/17.4	7.9/18.9	medium
S13	34.0	2.4/20.2	3.2/19.9	11.5/18.4	25.8/15.6	10.7/18.5	medium
S14	36.0	2.9/20.0	4.6/19.6	31.0/14.9	29.0/15.0	16.9/17.4	high
S15	23.5	5.4/19.4	10.0/18.4	26.5/15.2	33.4/14.5	18.8/16.9	high
S16	28.5	5.0/19.5	11.0/18.3	26.1/15.8	47.7/12.4	22.5/16.5	high
S17	27.0	11.0/18.0	10.4/18.4	21.9/16.4	50.6/11.5	23.5/16.1	high
S18	20.0	7.8/18.9	17.8/17.3	28.1/15.2	48.6/11.7	25.6/15.8	high
S19	17.0	6.8/19.2	12.0/18.0	31.8/14.8	61.0/10.1	27.9/15.5	high
S20	26.0	6.7/19.2	9.0/18.7	37.0/13.9	60.0/10.4	28.2/15.5	high
S21	26.5	9.1/18.7	17.3/17.5	20.1/15.6	68.7/8.0	28.8/14.9	high
S22	27.5	16.4/16.6	35.7/14.2	25.9/15.6	51.9/10.7	32.5/14.3	high

CO_2 stable isotope composition ($\delta^{13}C$ CO_2) was determined according to the procedures of Knohl et al. (2004) and Spötl (2004). Measurements were performed with a Europa Scientific 20-20 isotope ratio mass spectrometer (IRMS) with an ANCA-TG preparation module for trace gas samples. Stable isotope results are reported using the conventional delta-notation (δ), in per ml (‰) relative to the VPDB reference standard - $\delta^{13}C$ CO_2. The accuracy of the measurements was checked using "CO2 ISO-TOP, High" CO2 standard with $\delta^{13}C$ CO_2 value of 4.3 ± 0.2‰. The precision of measurements was ± 0.2‰.

2.3. Dendrochronological analysis

Cores were taken from 22 spruce trees (average age 36 years) at breast height (1.3 m) and perpendicular to the slope to avoid compression wood. Cores were air-dried, glued onto wooden holders, and sanded with progressively finer sandpaper until a highly polished surface was achieved (Stokes and Smiley, 1996). Samples were scanned using the ATRICS system (Levanič, 2007) and measured using WinDENDRO software. The width of each annual ring was measured to the nearest 0.01 mm. Chronologies were cross-dated with PAST-4™ software (www.sciem.com) using both on-screen visual comparisons and statistical parameters, such as the t-value after Baillie and Pilcher (tBP) (Baillie and Pilcher, 1973) and Gleichläufigkeit coefficient (GLK%) (Eckstein and Bauch, 1969). Quality control, checking for measurement errors, was performed using COFECHA software (Holmes, 1983), which is used for the assessment of measurement accuracy. It detects "outlier" ring measurements that lie in the outer portions ("tails") of the distribution of all rings for all series in a given year (Grissino-Mayer, 2001). These rings are flagged and listed in the output, and should be visually inspected and possibly re-measured to ensure that the original measurements are accurate (Grissino-Mayer, 2001).

2.4. Stable carbon isotope measurements in α-cellulose

α-cellulose was isolated from each pooled raw wood sample following the technique of Loader et al. (1997). Each sample was alternately treated for 48 h with $NaClO_{2(aq)}$ and $NaOH_{(aq)}$ solutions to remove lignin, hemicellulose, and other impurities. To ensure complete isotopic homogeneity within the pool, each extracted α-cellulose pool sample was homogenized for 60 s using a Hielscher UP200S (Teltow, Germany) ultrasonic probe (5-s pulses at 70% power) and freeze-dried for 48 h prior to analysis. Samples between 0.340 and 0.380 mg of dry α-cellulose were weighed into a tin foil capsule, crimped and placed into a sample tray and stored in a dry place until analysis.

Samples were analyzed for carbon isotopic composition using a Vario PYRO cube elemental analyzer (Elementar, Germany) interfaced with an IsoPrime100 isotope ratio mass spectrometer (IsoPrime, UK). Samples were combusted in an elemental analyzer at 950 °C with the presence of oxygen, converting nitrogen and carbon into N_2 and CO_2. Corrections and normalization to the international δ-scale were done manually. All samples were run in three replicates, and the reference materials were run in 4–10 replicate measurements within each run. Instrument performance and necessary instrument calibrations were performed prior to all analyses. Samples and reference materials used were prepared so as to yield signals of similar magnitudes. Stability and linearity checks prior to analysis yielded standard deviations of ≤0.04‰ and ≤0.01‰/nA, respectively. This is within the ≤0.08‰ standard deviation for stability and ≤0.03‰/nA for linearity specifications defined by the IRMS manufacturer.

2.5. Statistical analysis

Data of vertical CO_2-concentration profile measurements were first grouped for day- and night-time (6:00 to 18:00 and 18:00 to 6:00. respectively). They were averaged for each height and standard deviations were calculated.

Relations between air $[CO_2]$ and air isotopic C-signature ($\delta^{13}C$) were analyzed by means of a Keeling plot, in which $\delta^{13}C$ values were plotted against the inverse of $[CO_2]$ and linear regression was calculated yielding the R^2 value of a fit. In soil respiration studies, where accumulation chambers are used, this approach is a well-established technique to identify the isotopic character of soil CO_2 source (Chiodini et al., 2008). It yields a linear model, in which the intercept is the isotope ratio of soil CO_2 in the absence of dilution by atmospheric CO_2. We applied Keeling plots in the analysis of data obtained by free air sampling.

The difference between radial increments of trees growing in high and those growing in low CO_2 gas regimes was tested using a linear mixed model with the sampled trees as the random factor and time and the CO_2 regime as fixed factors. An autocorrelation term was also included in this model.

The analyses were performed in R 3.3.3 software (R Core Team, 2017).

3. Results

3.1. Gaseous environment and exposure of trees to geogenic CO_2

Averaged soil CO_2 concentrations ($[CO_2]_{soil}$) around the selected 22 mature spruce trees ranged from 0.2 to 32.5%. There were 6 trees where $[CO_2]_{soil}$ did not exceed the 3% level and were marked as *low CO_2 trees*. Medium-exposed trees (7 trees) were characterized by average soil concentrations ranging from 3.9 to 10.7% CO_2. Around these trees, the upper soil layer was characterized by close-to-control $[CO_2]_{soil}$ with a clear CO_2 enrichment at lower depths. For nine out of 22 trees, a rather high soil CO_2 enrichment was found. These *high CO_2 trees* showed average $[CO_2]_{soil}$ ranging from 16.9 to 32.5%. In some of these trees, $[CO_2]$ exceeded 10% in the upper soil horizon (depth of 10 cm; Table 1).

Continuous atmospheric CO_2 ($[CO_2]_{air}$) measurements along a height profile (P1 and P2 in Fig. 2) revealed clear CO_2 enrichment (Figs. 3 and 4a,b). At the "high soil CO_2" site, soil CO_2 concentrations (depth 0.3 m) exceeded the measuring range of analyzer ($> 20000\ \mu mol\ mol^{-1}$), whereas they reached $3300\ \mu mol\ mol^{-1}$ (day) and $3400\ \mu mol\ mol^{-1}$ at the "low soil CO_2" site. At the soil surface of the "high soil CO_2" site, the average $[CO_2]_{air}$ was 1690 and $1840\ \mu mol\ mol^{-1}$ during day and night, respectively, while much lower average values were detected at "low soil CO_2" site (650 and $750\ \mu mol\ mol^{-1}$). The atmosphere was characterized by highly variable CO_2 concentrations (Fig. 3). A diurnal pattern with higher nocturnal $[CO_2]_{air}$ was observed, and a more stable gas regime was detected during a rainy day (30 Sept). At the "low soil CO_2" site, the average $[CO_2]_{air}$ at the height of 1.3 m was $574\ \mu mol\ mol^{-1}$ during the day and $614\ \mu mol\ mol^{-1}$ during the night. At the "high soil CO_2" location, the average $[CO_2]_{air}$ at the height of 1.3 m was $618\ \mu mol\ mol^{-1}$ during the day and slightly higher during the night ($664\ \mu mol\ mol^{-1}$). Rather unexpectedly, a different situation was found in the crown layer (height 5–20 m) where the concentrations were higher in the "low soil CO_2" than in the "high soil CO_2" location (Fig. 4). At 20 meters $[CO_2]_{air}$, this difference was 561 vs. $476\ \mu mol\ mol^{-1}$ during the day, 596 vs. $507\ \mu mol\ mol^{-1}$ during the night (Fig. 4a and b).

Air emitted from the main vent had a $\delta^{13}C$ CO_2 value of −3,45‰, while the value for air sampled at profiles ranged from −9.87 to −2.98 (−1.64) ‰. Figures showing $[CO_2]$ and $\delta^{13}C$ CO_2 profiles at two mature trees (Fig. 4c and d) reveal that geogenic CO_2 contributes to the enrichment of lower atmospheric layers (Fig. 4d, surface and height of 1.3 m). $\delta^{13}C$ CO_2 values indicate that geogenic CO_2 could also contribute to high CO_2 concentrations that were observed in the crown layer at the "low soil CO_2" location. In contrast, at the "high soil CO_2" location, air at 5 m height and above was characterized by relatively uniform $\delta^{13}C$ CO_2 values and $[CO_2]$, which demonstrates that in this case atmospheric enrichment is limited to lower layers only.

Additional gas measurements were made close (2–5 m) to the main vent (P3 on Fig. 2). A substantial enrichment of the atmospheric CO_2 was observed. Close to the ground (10 cm), average $[CO_2]$ was above $3000\ \mu mol\ mol^{-1}$ during the day and well above $4000\ \mu mol\ mol^{-1}$ during the night. At the highest measuring point (70 cm), average daily and night CO_2 concentrations were 1280 and $1700\ \mu mol\ mol^{-1}$, respectively (Fig. 5, a). CO_2 enrichment patterns corresponded well with high $\delta^{13}C$ CO_2 values (Fig. 5, b), which indicates that the air is enriched by mofettogenic CO_2. Enrichment was higher during the night-than during the daytime. The contribution of mofette air to CO_2 enrichment was furthermore corroborated when carbon signature and CO_2 concentrations (both analyzed in the same samples by IRMS) were compared.

Data from all profiles were combined, comparing $\delta^{13}C$ CO_2 and reciprocal $[CO_2]_{air}$, in a manner of a Keeling plot. The slope of the obtained linear model was, in contrast to usual soil respiration Keeling plots, negative (Fig. 6). The values for all samples fit very well to the model. This both indicates that geogenic CO_2 strongly dominates in contributing the soil CO_2, and (after degassing) also to atmospheric CO_2, completely overriding the respiratory (i.e. biogenic) soil CO_2 source. Therefore, the intercept of our linear model shows an isotope ratio of geogenic (mofette) CO_2. This value is −3.51‰, which is close to the $\delta^{13}C$ of the air sampled directly at the vent (−3.45‰).

3.2. Tree growth

Analyses of tree ring widths showed that the studied spruce trees are of similar age, approximately 40 years old. Measurements of tree-ring widths reveal a general decrease in growth increment over the years, which is typical for trees that have not yet achieved full maturity (Fig. 7). The first years are characterized by wider rings, while in later years the water transport function can be efficiently maintained by narrower rings. All trees responded similarly to dry years during their growth period (1976, 1993, and 2003). Furthermore, higher growth increment in trees from control locations was found, while trees exposed to higher soil CO_2 concentrations revealed smaller annual increments (Fig. 7). A weak but significant correlation ($R^2 = 0.18$) was found between soil $[CO_2]$ at the depth of 20 cm and stem diameter increment in the period 2002–2011.

Isotopic analyses showed that the wood of trees which grew at control plots ($[CO_2]_{soil} < 3\%$) was more depleted in ^{13}C than in trees exposed to elevated geogenic CO_2 (Fig. 8). In most trees, $\delta^{13}C$ is slightly decreasing (values are less negative) over time.

4. Discussion

The gas regime of mofettes has been most commonly described based on instantaneous atmospheric CO_2 measurements made by IR-analyzers or absorptive diffusion tubes. This approach has its limitation when temporal variations of $[CO_2]_{air}$ are addressed to estimate long-term average CO_2 enrichment and/or when plants differently exposed to geogenic CO_2 are selected for experimentation. Short-term fluctuations of $[CO_2]_{air}$ within mofette areas can be described by using automated sampling systems coupled with IR analyzers (van Gardingen et al. 1995). Continuous measurements were recently applied to describe CO_2 accumulation patterns in the Bossoleto mofette (Kies et al., 2015). In our research, we combined short-term continuous measurements in the atmosphere with measurements of soil CO_2, which had been found to correlate well with growth and physiological parameters of mofette plants and can be hence regarded as a good measure of their average CO_2 exposure (Pfanz et al., 2004). Soil CO_2 concentrations at Stavešinske slepice mofette were in a similar range as known for other mofettes in the region (Vodnik et al., 2006, 2009) and elsewhere (Saβmannshausen, 2010; Thomalla, 2015). About 25% of the area of interest shows hypoxic conditions.

In mofette areas, not only the soil but also the air can be enriched with geogenic CO_2. In depressions or valleys, gas lakes may form, whereas in flatter areas wind, convection and advection help to evenly distribute the gas. In forests, some CO_2 accumulation can be expected because of the wind-tranquilizing effect of canopy and stems. However, in lower air layers, CO_2 accumulation is expected to be much more pronounced than in higher layers. Atmospheric CO_2 enrichment was, however, not limited only to layers close to the ground. CO_2 concentrations measured in the middle and upper canopy were relatively high in comparison to literature data. In the canopy of a tropical forest $[CO_2]_{air}$ was found to be around $450\ \mu mol\ mol^{-1}$ during nights (de

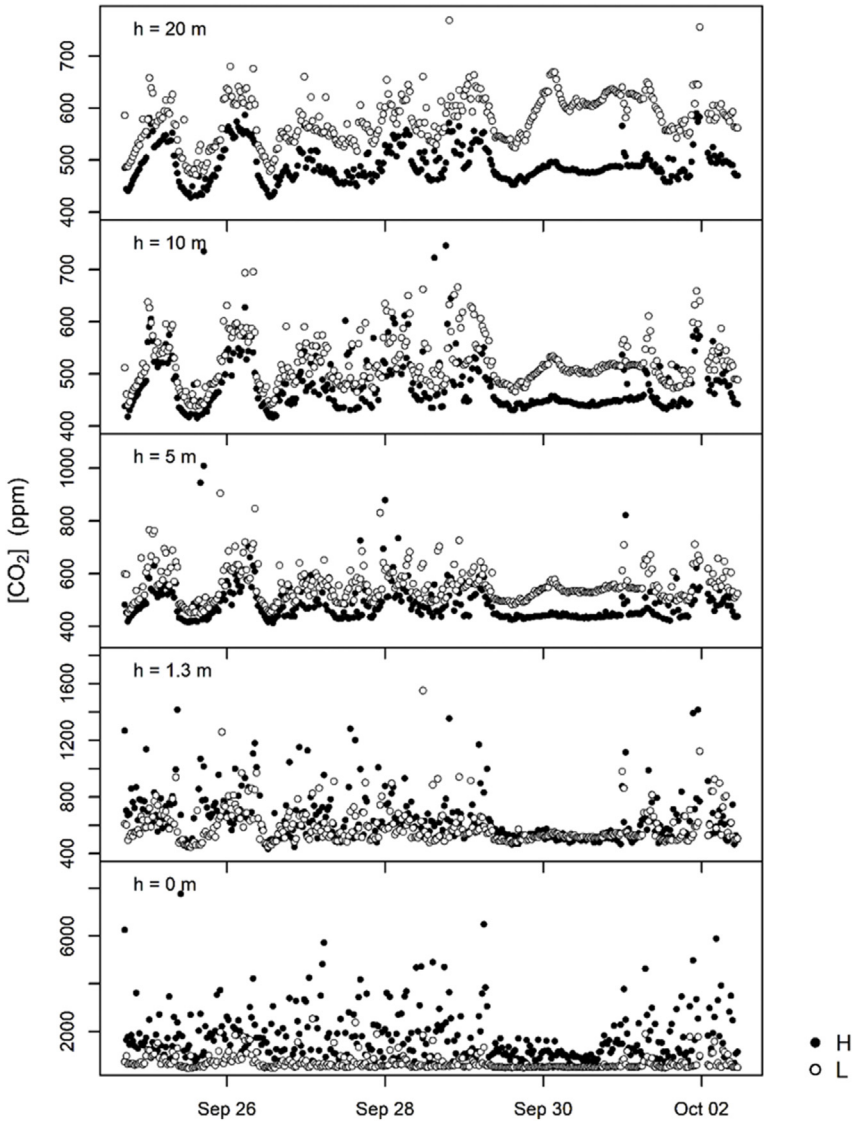

Fig. 3. Atmospheric CO_2 concentrations in the air sampled at different heights (h = 0–20 m) along vertical profiles (P1, P2, Fig. 2) set at the "low soil CO_2" (L), where soil CO_2 concentration was in the range typical for non-mofette soil, and at the "high soil CO_2" location (H), with substantial CO_2 enrichment in the soil.

Araújo et al., 2008) and very high nocturnal concentrations were also observed in boreal forest dominated by Scots pine and Norway spruce (Feigenwinter et al., 2010) where $[CO_2]_{air}$ reached values close to 500 µmol mol^{-1}. These measurements revealed nearly uniform vertical CO_2 profiles throughout the lower and middle canopy and a sharp vertical gradient in the upper part of the canopy. In our case, similar concentrations were detected in mid-canopy (height 5–15m) on both plots. In contrast to reports from sites with no geogenic enrichment, these high concentrations were, however, not limited to the nocturnal period, but also persisted during the day. Usually, the daily $[CO_2]_{air}$ within the canopy is reduced by photosynthetic assimilation and air movements; it can decrease close or even below ambient CO_2 concentration (de Araújo et al., 2008). Due to the presence of mofette gas, a fairly stable (in a nine-day measuring period for CO_2) enrichment of air within the spruce canopy was established. Measurements in the upper part of the canopy showed spreading of CO_2-enrichment to locations further from gas-releasing vents. It can be assumed that the distribution of geogenic CO_2 within the canopy results from diffusion, driven by steep concentration gradients, and largely from vertical and horizontal advective movements.

Due to the highly dynamic nature of the atmosphere, insight into spatial and temporal patterns is very limited. Thus far, data on the temporal variability of $[CO_2]_{air}$ have been published only for the Bossoleto mofette in Italy, which is a bowl-like depression (sinkhole) with gas-releasing vents at the bottom of the valley. The continuous degassing results in distinct temporal gas enrichment patterns (vertical gradients), which can be more easily followed than in forest sites (van Gardingen et al. 1995; Kies et al., 2015). For more complicated topography, e.g. flat areas, description of CO_2 enrichment in the canopy could be obtained by more detailed spatial information about the canopy structure and CO_2 distribution. However, research by Feigenwinter and coworkers (Feigenwinter et al., 2010), which used five 30-m tall towers in 60 × 60 m area, revealed that this is not an easy task even at the sites with no geogenic CO_2 enrichment.

Similarly, difficulties are encountered when describing the stable isotopic composition of canopy air within the area. At different locations and in different time very variable $\delta^{13}C$ can be expected due to a different mixing ratio between CO_2-enriched sites and background air. Saurer et al. (2003) estimated effective yearly canopy air $\delta^{13}C$ at the Lajatico mofette on the basis of ^{14}C-calculated CO_2 concentration in the canopy, relying on ^{14}C signatures of tree rings. Calculated values of $\delta^{13}C$ in the air at high CO_2 locations ranged from −9.0 to −8.5‰ and were closer to the $\delta^{13}C$ value of pure mofette venting air (−9.1‰) than values for the air from low CO_2 locations. Compared to these reports,

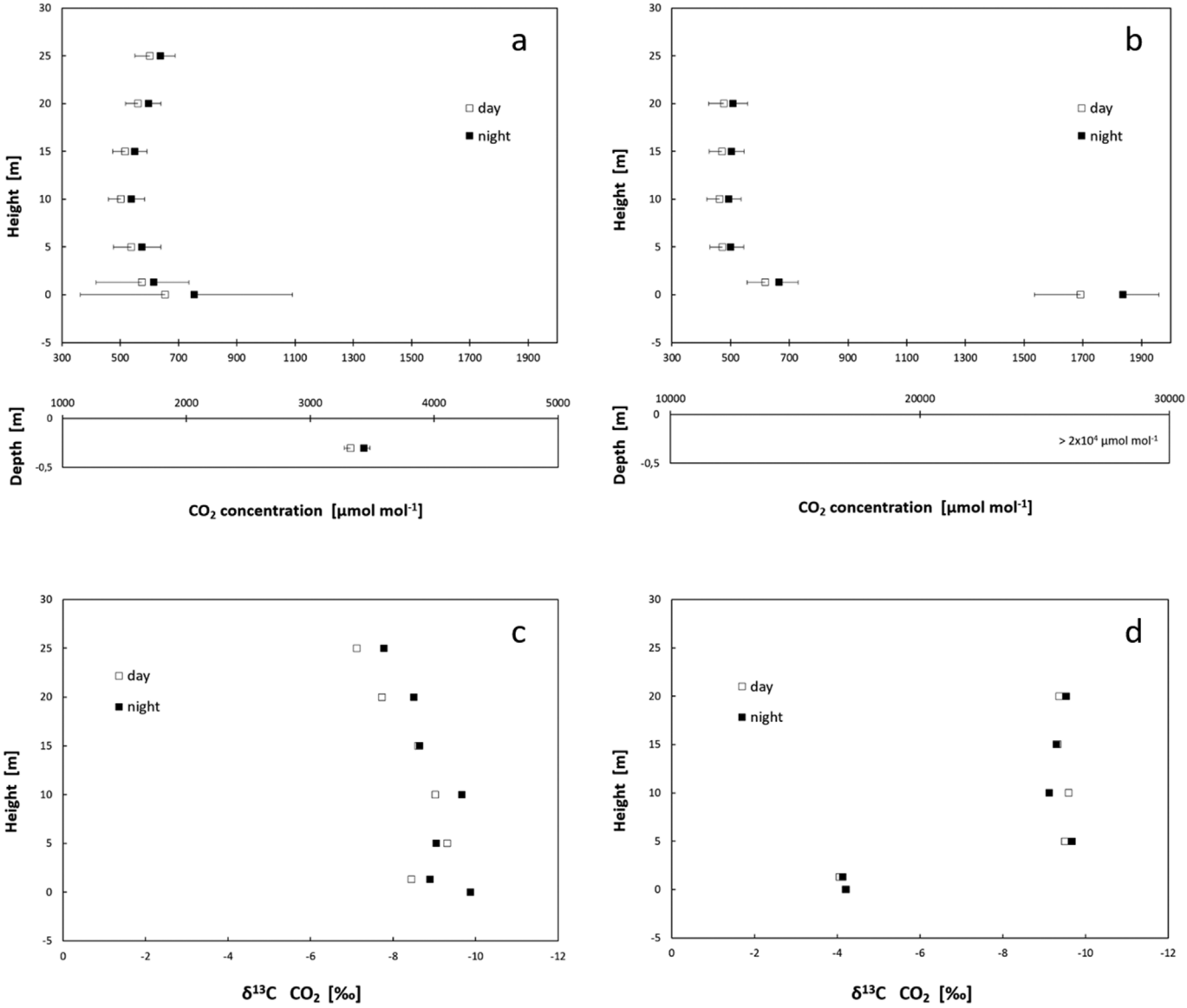

Fig. 4. Atmospheric CO_2 concentrations (a, b) and corresponding CO_2 stable isotope composition ($\delta^{13}C$ CO_2; c, d) along vertical profiles set at the "low soil CO_2" location (a, c), where soil CO_2 concentration was in the range typical for non-mofette soil, and at the "high soil CO_2" location, with substantial CO_2 enrichment in the soil (refer to Table 1). Day (8:00–18:00) and night (18:00–6:00) data were averaged for the measuring period (24 Sept. 2013 to 2 Oct. 2013). Bars in a and b represent standard deviations.

the most discernable vents of the Stavešinske slepice mofette are characterized by CO_2 less depleted in ^{13}C. In a wider surrounding, SW Prekmurje region, which is known for geothermal activity, the signature of degassed CO_2 varies from −6.1 to −2.2‰ (Pezdič; Žižek, 1993). For a nearby meadow mofette in Stavešinci, the $\delta^{13}C$ of CO_2 in the gas from the vent was −3.9‰ (Vodnik et al., 2002). On the basis of measurements of mofette venting air and our Keeling plot analysis (Fig. 6), we estimate the $\delta^{13}C$ of Stavešinske slepice mofette to be −3.5‰. The high and significant correlation between the $\delta^{13}C$ of the atmospheric CO_2 and its reciprocal of its concentration ($1/[CO_2]_{air}$) proves that enrichment originates from the geogenic source. This can be seen not only for air layers close to the ground but also for the upper crown layer at the "low soil CO_2" location. This proves that CO_2 enrichment, detected there, does not originate from respiratory but from geogenic CO_2 sources.

Higher $\delta^{13}C$ values in the wood of "high soil CO_2 trees" can be related to the soil and atmospheric gas conditions. First, trees are known to respond to long term hypoxia (e.g. induced by flooding) by reduced photosynthesis, decreased transpiration and stomatal conductivity (Pezeshki et al., 1996). As a result of the reduced stomatal conductivity and low intracellular CO_2 concentrations, photosynthetic enzymes (Rubisco) discriminate less against ^{13}C, thus mitigating photosynthetic limitation. Such a response could be also expected in trees growing in high CO_2 (low O_2) soil and would manifest in higher $\delta^{13}C$ values of plant biomass. It is, however, not very likely that mofette trees actually lack carbon dioxide. The research by Tognetti et al. (1998) showed separation in the relationship between photosynthesis and leaf conductance in *Q. pubescens* and *Q. ilex* trees growing near a mofette. Despite reduced stomatal conductivity induced by high $[CO_2]_{air}$ (and possibly by soil hypoxia), these trees preserved high photosynthetic rates, which is evidence against CO_2 insufficiency in the leaves. It can be presumed that high concentrations of CO_2 in the air support higher diffusive flow, which is expected to bring enough photosynthetic substrate into the leaf. Despite the unknown mixing ratio between atmospheric and mofette air at Stavešinske slepice mofette, it is clear that air in the canopy has a substantially increased fraction of ^{13}C. Hence, it could be expected that mofette trees assimilate a greater fraction of ^{13}C than trees growing in non-enriched areas.

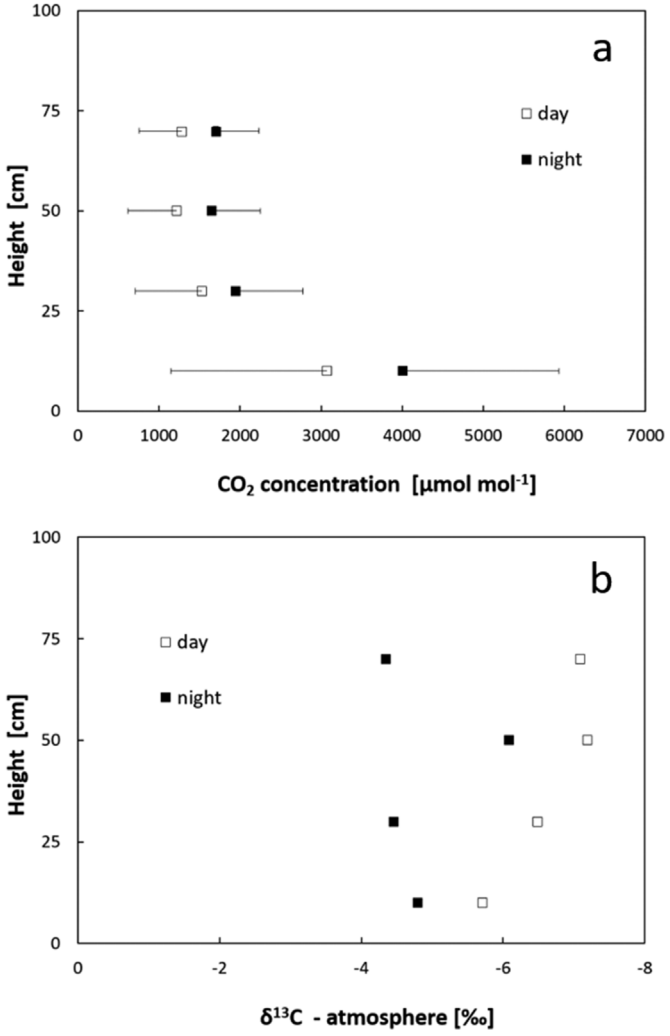

Fig. 5. Atmospheric CO_2 concentrations (a) and corresponding CO_2 stable isotope composition ($\delta^{13}C$ CO_2; b) along vertical profiles set at the young spruce trees close to the main vent of Stavešinske slepice mofette. Day (8:00–18:00) and night (18:00–6:00) data were averaged for the measuring period (2.10.2013 to 10.10.2013). Bars in (a) represent standard deviations.

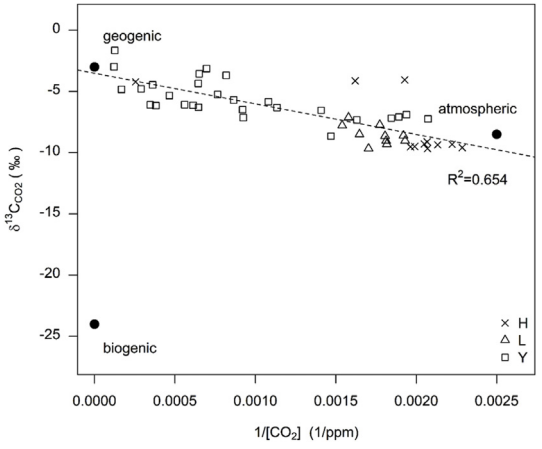

Fig. 6. Stable carbon isotope composition ($\delta^{13}C$) of air CO_2 versus reciprocal of $[CO_2]_{air}$ (Keeling plot). Signature estimates of CO_2 in non-mofette atmospheric air (atmospheric), soil respired air (biogenic), and mofette air (geogenic) are plotted as black circles. Data from profiles set at the "high soil CO_2" location (H), "low soil CO_2" location (L) and at the young spruce trees close to the main vent of Stavešinske slepice mofette (Y) are shown.

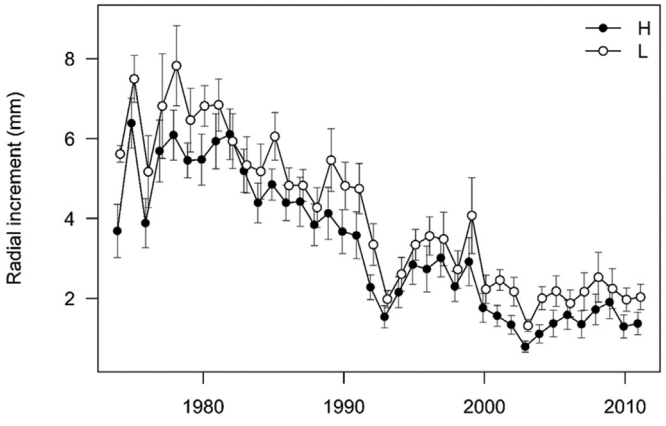

Fig. 7. Radial growth of Norway spruce (*Picea abies* (L.) Karsten) trees growing at Stavešinske slepice mofette. Mean radial increments ± standard deviations are shown in time for trees exposed to low- (L) and high soil CO_2 (H) concentrations (refer to Table 1, for their CO_2 exposure), N = 6–9.

Irrespective of soil CO_2 regime, the $\delta^{13}C$ of the wood increases with the age of the trees. This trend can be explained by a "juvenile effect", i.e. depleted but rising values in the early years of growth. It has been variously attributed to the incorporation of respired CO_2 and to changes in hydraulic conductivity as trees gain height (Gagen et al., 2007).

The growth of spruce at the tested area was shown to be influenced by the prevailing soil gas regime. Trees growing at high $[CO_2]_{soil}$ had a lower radial growth rate, despite being exposed to atmospheric CO_2 concentrations which could promote carbon assimilation and water use efficiency. This is not in accordance with some other studies (Hättenschwiler et al., 1997; Vejpustkova et al., 2016) that explain that growth of mofette trees can be enhanced by elevated CO_2. In drought-prone ecosystems, the positive effect could be largely attributed to the beneficial effect on water relations (Hättenschwiler et al., 1997). Plants exposed to atmospheric CO_2 enrichment can reduce stomatal conductivity and increase water use efficiency, which is translated into better growth. However, no such effect was found in the study of Mediterranean woody plant community growing in a mofette area, despite being water-limited (Tognetti et al., 2000a,b). It is suggested that other resource limitations, such as low nutrient availability (common in the Mediterranean region), may have counteracted the positive effect of elevated CO_2 under drought stress, or trees may have acclimated to the high CO_2.

There is another report on positive effects from temperate climate. Growth variations between aspen trees (*Populus tremula* L.) growing on a CO_2-enriched mofette site were studied in the floodplain of the Plesná Stream near Hartoušov, Czech Republic (Vejpustkova et al., 2016). Similar to our study, the exposure of trees was estimated on the basis of $[CO_2]_{soil}$. Cumulative stem basal area growth of high- and low-$CO_{2\ soil}$ trees started to diverge at the age of ten years. At the age of 25 years, the basal area of the high-$CO_{2\ soil}$ trees exceeded the basal area of the low-CO_{2soil} trees by 39%. Height growth exhibited similar dynamics to that of the basal area up to the age of 25 years; thereafter it slowed down. The authors conclude that geogenic CO_2 promotes growth by photosynthetic enhancement. They presume that CO_2 transported from the soil via roots and stem could increase photosynthetic activity. Indeed, CO_2 transported in the xylem sap can be assimilated in photosynthesizing leaf and stem cells (McGuire et al., 2009; Bloemen et al., 2013). In addition, there is extra CO_2 which is transported via the gaseous pathway of the apoplast by diffusion which could contribute to leaf carbon fixation. However, a clear growth promoting effect can be expected only in the case that $[CO_2]$ is a limiting factor for growth. In most mofettes, this is not the case. Very frequently there is a lack of mineral nutrients due to decreased availability resulting from changed soil conditions, i.e. low pH and redox potential (Pfanz et al., 2004).

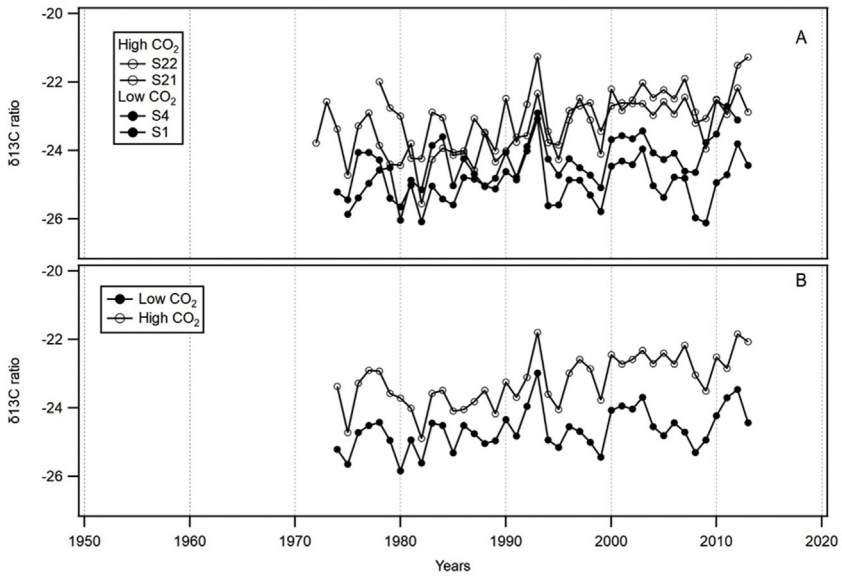

Fig. 8. Stable carbon isotope composition ($\delta^{13}C$) of tree rings of four Norway spruce trees (S1, S2, S15, S16) growing at different levels of soil CO_2 enrichment at the Stavešinske slepice mofette. Soil CO_2 concentrations (depth 0–60 cm) around the trees were: S1 = 32.5%, S2 = 28.8%, S15 = 1.6% and S16 = 0.2%.

Moreover, high soil CO_2 can also impair root functions as shown for root respiration of herbaceous plants (Maček et al., 2005), which can additionally limit the uptake of nutrients. Therefore, we believe that growth promotion can be found only exceptionally. In the case of aspen trees stand at Hartoušov, possible inputs of mineral nutrients from nearby managed grassland cannot be excluded (Pfanz, personal communication). Significant growth enhancement of aspen can also be related to the great growing potential of this pioneer woody species.

These results show that the effects of geogenic CO_2 on mofette vegetation cannot be fully generalized. They are strongly dependent on regional, local CO_2 enrichment patterns, which are related to the strength of geogenic source, parent rock/soil characteristics and topography. Furthermore, they depend on vegetation properties (age, phenology, tolerance to hypoxia and soil acidity, nutritional status etc.) and can be modified by other environmental factors (e.g. water deprivation).

5. Conclusions

Our research reveals that mofette-grown trees sense geogenic CO_2 at different levels. First, their roots experience changed soil gaseous conditions with high $[CO_2]$ and low $[O_2]$. Previous research has shown that this impairs mineral nutrition due to the reduced availability of essential nutrients (e.g. nitrogen) or exposed toxicity (e.g. Mn^{2+}, Fe^{2+}, H_2S) under changed pH and redox potential of the soil (Elzenga and van Veen, 2010). While direct effects of elevated CO_2 on roots are expected only at the very enriched sites, close to the vents (Maček et al., 2005), a spatially spread, CO_2-induced soil hypoxia could have profound inhibitory effects on root growth (Armstrong and Drew, 2002), contributing to changed root architecture where roots “avoid” hypoxic soil. In shallow-rooting tree species growing in a non-homogeneously degassing soil such an avoidance could weaken mechanical stability, which is indicated by field observations of tree positions at different mofettes (Pfanz and Vodnik, unpublished).

Gas measurements along vertical profiles revealed that the young trees close to the spring are exposed to $[CO_2]_{air}$ as high as 1500 μmol mol^{-1}. Other research has shown that such CO_2 enrichment does not have negative effect on photochemical efficiency of spruce needles (Vodnik and Eler, unpublished). In contrast, the possible fertilizing effects of CO_2 are questionable due to nutrient limitations. This indicates that more or less neutral (or at least inconsistent) effects of CO_2-enriched atmosphere on the crowns of young trees could be expected. However, nothing is known how elevated $[CO_2]_{air}$ in the lower air layers could affect stem physiology of young and older trees.

We have shown that the crowns of mature trees can still sense elevated CO_2 in the concentration range, which is expected for mid-century according to global change scenarios (IPCC, 2013). In case they are not exposed to the changed gaseous conditions in the soil, possible positive effects of CO_2 enrichment could not be excluded. However, such effects are expected to be only sporadic and are probably of limited ecological relevance. In contrast, unfavorable soil gas conditions should be generally accepted as by far most important factor affecting (i.e. disturbing) the growth of mofette trees.

Acknowledgements

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